IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

T. William Hutchens *et al.*

Application No.: 10/626,301

Filed: July 23, 2003

For: RETENTATE CHROMATOGRAPHY AND PROTEIN CHIP ARRAYS WITH APPLICATIONS IN BIOLOGY AND MEDICINE

Customer No.: 53671

Confirmation No. 1861

Examiner: Teresa D. Wessendorf

Technology Center/Art Unit: 1639

DECLARATION UNDER 37 C.F.R. § 1.132 OF TAI-TUNG YIP

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Tai-Tung Yip, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I am currently a Director of Research and Senior Research Fellow at Ciphergen Biosystems, Inc., Fremont California. Prior to that, I have held the following positions:

Associate Biochemist, University of California at Davis;

Assistant Professor, Baylor College of Medicine, Houston, Texas.

3. I hold a Ph.D. in Biochemistry from the Chinese University of Hong Kong.

4. My current research interests are proteomics, in particular using mass spectrometry. As one of the pioneering developers of SELDI, I have numerous patents in these disciplines along with many publications and presentations. My *Curriculum Vitae* is attached as Exhibit A.

5. I am an inventor on the above-referenced patent application, I have reviewed it, and I am familiar with the contents therein.

6. I have reviewed the specification and the presently pending claims for the above-referenced application.

7. The presently claimed methods for detecting translation of an mRNA can be carried out methodically and predictably without undue experimentation.

8. Attached to this Declaration as Exhibit B is an example of an experiment, wherein a protein translated in a cylindrical well attached to a mass spectrometry substrate was methodically and predictably bound to an adsorbent and then detected by mass spectrometry.

9. The example attached to this Declaration teaches all of the steps of the claimed methods for the present application:

a) providing a substrate for use in desorption spectrometry, wherein the substrate comprises a surface and an adsorbent attached to the surface;

b) providing an mRNA encoding a polypeptide and reagents for translation of the mRNA;

c) translating the mRNA *in situ* on the substrate, whereby the polypeptide is produced and is bound through the adsorbent to the substrate;

d) exposing the substrate to an eluant to wash off unbound material and to allow retention of the polypeptide by the adsorbent; and

e) detecting retained polypeptide by desorption spectrometry.

10. The example attached to this Declaration differs from the pending claims of the present application in that in the example, the protein is translated in an *E. coli* cell, and in the claims, the protein is translated *in vitro*. However, protocols for *in vitro* translation were well known and commonly practiced at the time of the June 20, 1997 priority date of the present application. Kits were readily purchasable from, for example, Promega of Madison, Wisconsin at that time.

11. In view of the teachings provided in the specification and the Example attached as Exhibit B, it is my opinion that the presently claimed methods for detecting translation of an mRNA can be carried out methodically and predictably without undue experimentation.

The declarant has nothing further to say.

Tai-Tung Yip _____ Date _____

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Tai-Tung Yip	**Director of Research** **Senior Research Fellow**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
The Chinese University of Hong Kong, Hong Kong	B.S.	1969	Biology
The Chinese University of Hong Kong, Hong Kong	Ph.D.	1985	Biochemistry
Biomedical Center, University of Uppsala, Sweden	Postdoctoral	1986	Biochemistry & Chemistry

Professional Experience:

1987 – 1988	Instructor, Department of Pediatrics, Baylor College of Medicine, Houston, Texas
1988 – 1993	Assistant Professor, Department of Pediatrics, Baylor College of Medicine, Houston, Texas
1994 – 1996	Associate Biochemist, Department of Food Science & Technology, University of California, Davis
1997-present	Director of Biology Research, Senior Research Fellow, Ciphergen Biosystems Inc., Fremont, California

Honors and Awards:

1993 - present	Co-inventor of Surface Enhanced Laser Desorption Ionization Mass Spectrometry Over 10 issued patents

Recent Publications:

1. **Yip TT**, Lomas L. SELDI ProteinChip array in oncoproteomic research. Technol Cancer Res Treat. 2002;1(4):273-80

2. Poon TC, **Yip TT**, Chan AT, Yip C, Yip V, Mok TS, Lee CC, Leung TW, Ho SK, Johnson PJ. Comprehensive proteomic profiling identifies serum proteomic signatures for detection of hepatocellular carcinoma and its subtypes. Clin Chem. 2003;49(5):752-60

3. Cho WC, Yip TT, Yip C, Yip V, Thulasiraman V, Ngan RK, **Yip TT**, Lau WH, Au JS, Law SC, Cheng WW, Ma VW, Lim CK. Identification of serum amyloid a protein as a potentially useful biomarker to monitor relapse of nasopharyngeal cancer by serum proteomic profiling. Clin Cancer Res. 2004;10(1 Pt 1):43-52.

4. Thulasiraman V, Wang Z, Katrekar A, Lomas L, **Yip TT**. Simultaneous monitoring of multiple kinase activities by SELDI-TOF mass spectrometry. Methods Mol Biol. 2004;264:205-14.

5. Yip TT, Chan JW, Cho WC, **Yip TT**, Wang Z, Kwan TL, Law SC, Tsang DN, Chan JK, Lee KC, Cheng WW, Ma VW, Yip C, Lim CK, Ngan RK, Au JS, Chan A, Lim WW. Protein Chip Array Profiling Analysis in Patients with Severe Acute Respiratory Syndrome Identified Serum Amyloid A Protein as a Biomarker Potentially Useful in Monitoring the Extent of Pneumonia. Clin Chem. 2005;51(1):47-55.

6. Wang Z, Yip C, Ying Y, Wang J, Meng XY, Lomas L, **Yip TT**, Fung ET. Mass spectrometric analysis of protein markers for ovarian cancer. Clin Chem. 2004;50(10):1939-42.

EXHIBIT B

EXAMPLE 1: On-chip Monitoring of recombinant His-PelleC protein expression in *E. coli* by SELDI

Cell culture and expression system

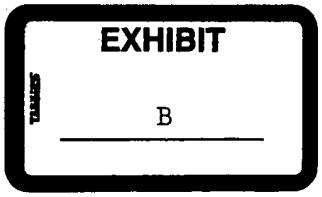
IPTG inducible vector pQE30 (Qiagen) was used to clone the His-PelleC gene. The protein was expressed in *E. coli*. M15 cells. An overnight culture of *E. coli* cells containing the expression plasmid of interest was grown up in 5 ml of LB/antibiotics at 37°C. The culture was diluted 1:10 in fresh LB/antibiotics before loading onto chips.

Preparation of IMAC-3-Ni chips and on-chip growth

IMAC-3 chips were charged with 5 µl of 50mM nickel chloride for 5 min each, twice, followed by rinsing with 5 µl of water once and 5 µl of PBS twice for 5 min each. Load the chips in a 96-well format bio-processor. In each well, aliquot diluted cell culture in a volume varying from 10 µl to 125 µl. The cells were grown for 1.5 hrs at 37°C until OD_{600} reaches 0.6. IPTG was added to a final concentration between 0 to 1 mM. The cells were grown for an additional 1-3 hrs at 37°C to allow protein expression. A schematic diagram is outlined in Figure 1.

Lysis of cells and binding of His-tagged protein to IMAC-Ni surface

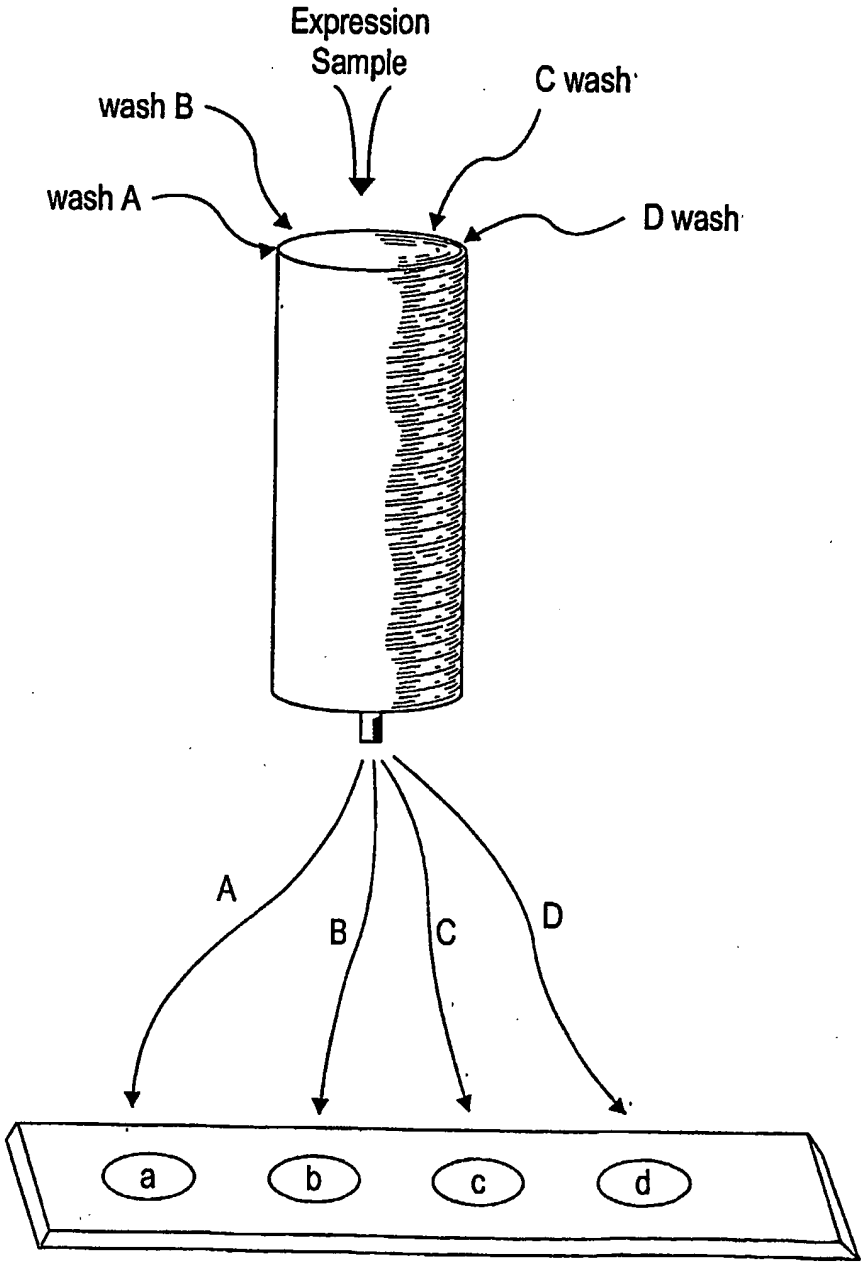
At the end of induction, the cells were spun down in the bio-processor at 2,000 rpm for 10 min at 4°C and medium was removed from each well. Ten micro liters of a buffer containing 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole and 1 mg/ml lysozyme, pH 8 was added to each well. The cells were lysed for 30 min before 90 µl of the buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole) was added to dilute out the lysozyme. Continue incubation for 1 hr to allow binding of His-tagged protein to the surface (Figure 6).



Alternatively, at the end of induction, the cells were not centrifuged, instead a 10x solution containing 10 mg/ml lysozyme was added directly to the medium in the wells to lyse the cells for 30 min. Continue to incubate in the same medium for 1 hr.

At the end of 1 hr, the solution was removed, and 100 µl of a buffer containing 50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole and 0.05% Tween-20, pH 8 was added to each well to wash for 5 min, 3 times. The chips were then rinsed with water and allowed to dry. Energy-absorbing matrix (EAM) was added to spots. The chips were ready for MS analysis. Broth volume optimization is shown in Figure 3 while a comparison of the two methods of *E. coli* disruption are shown in Figure 4.

FIG. 1



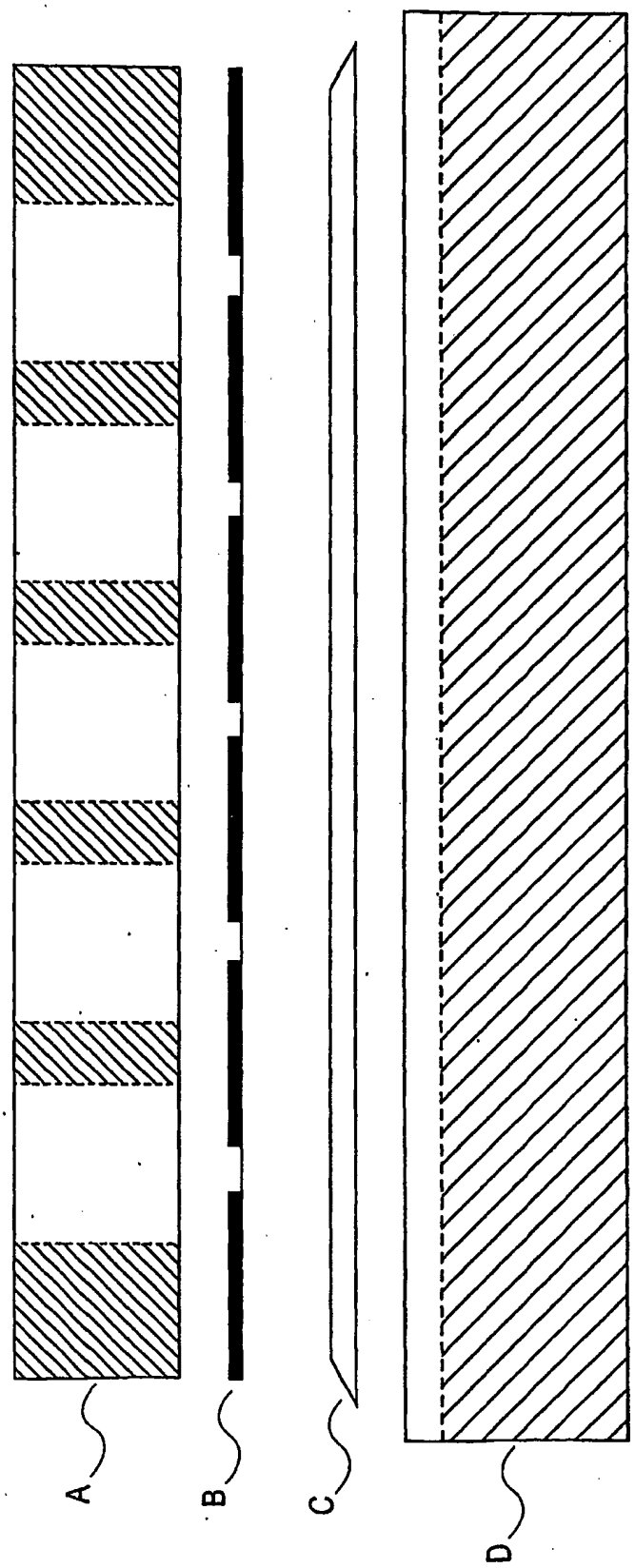


FIG. 2

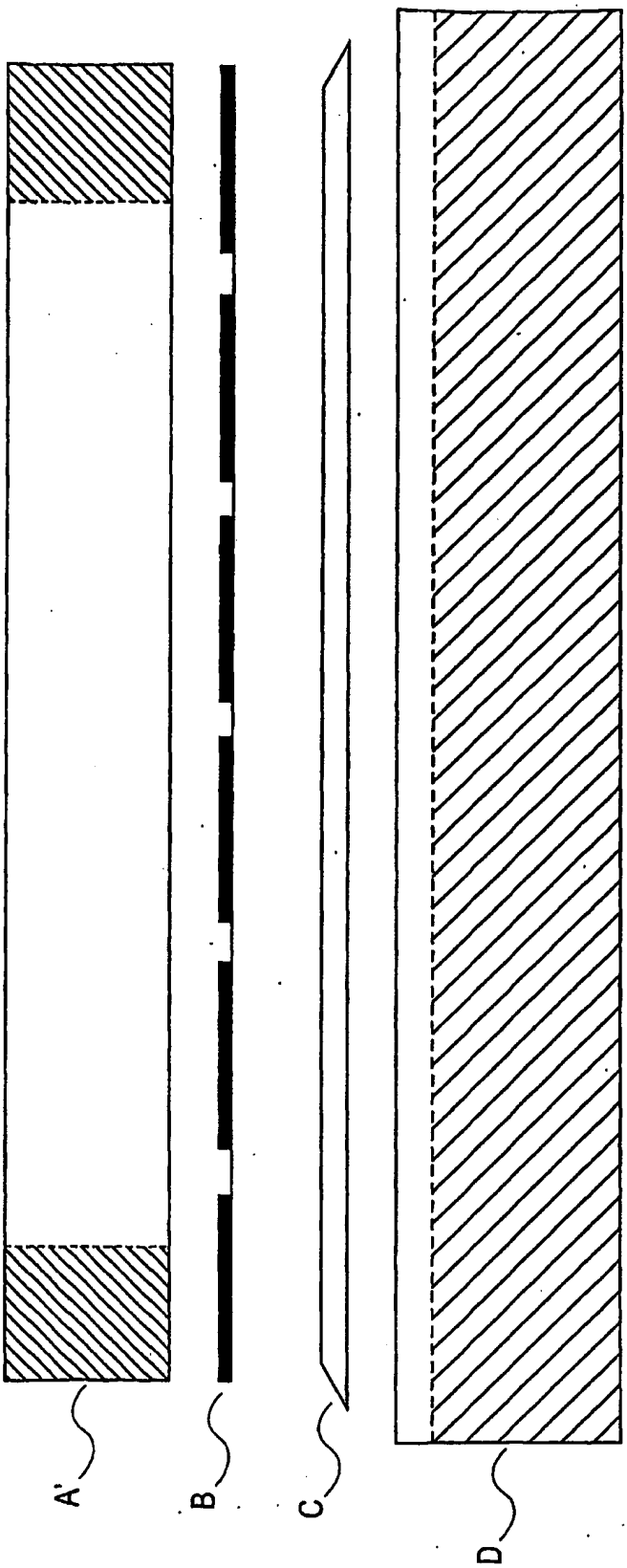


FIG. 3

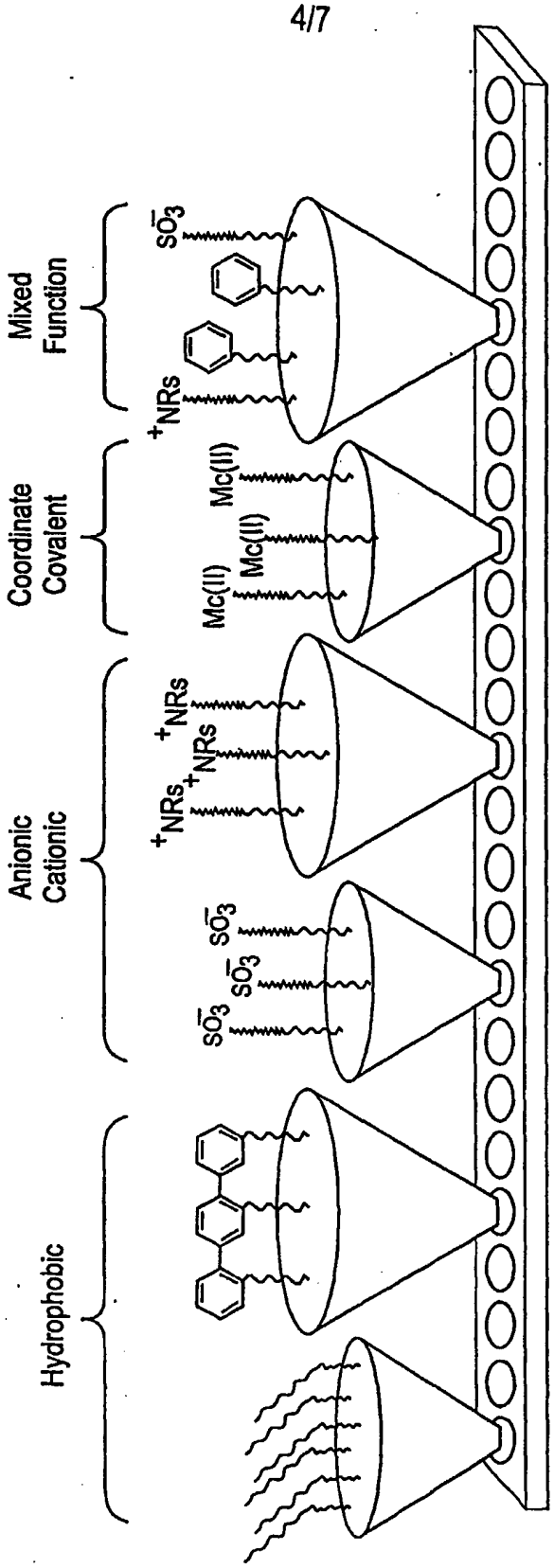

Mixed Function
Coordinate Covalent
Anionic Cationic
Hydrophobic
SO_3^-
^+NRs
Mc(II)

FIG. 4

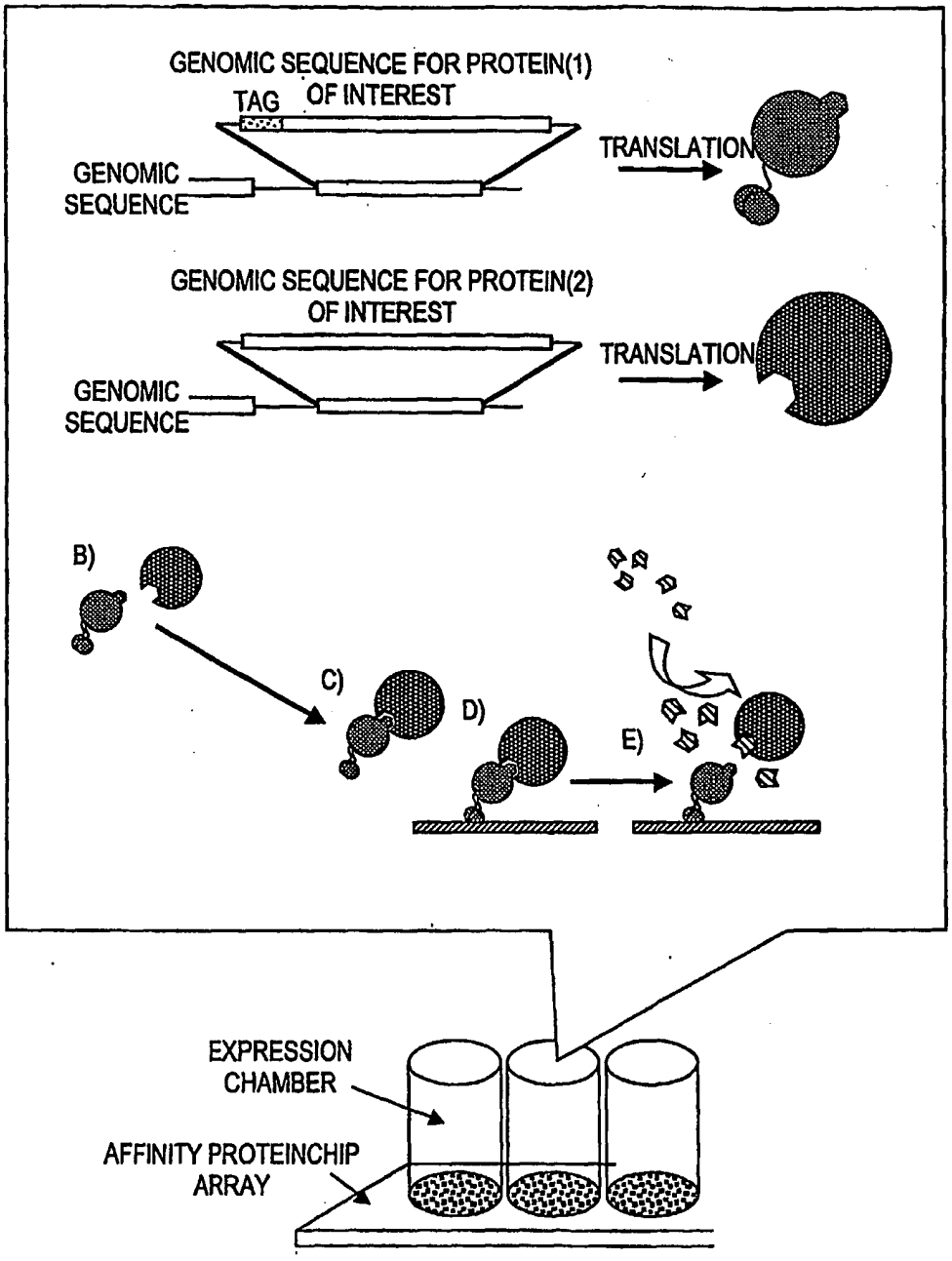
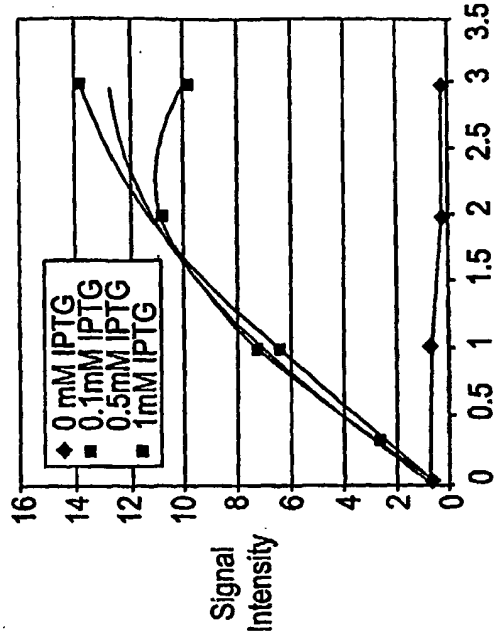
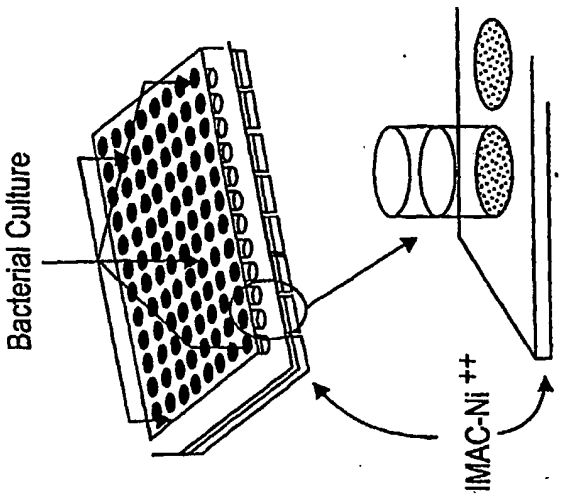

GENOMIC SEQUENCE FOR PROTEIN(1)
TAG
OF INTEREST
GENOMIC SEQUENCE
TRANSLATION
GENOMIC SEQUENCE FOR PROTEIN(2)
OF INTEREST
GENOMIC SEQUENCE
TRANSLATION
B)
C)
D)
E)
EXPRESSION CHAMBER
AFFINITY PROTEINCHIP ARRAY

FIG. 5



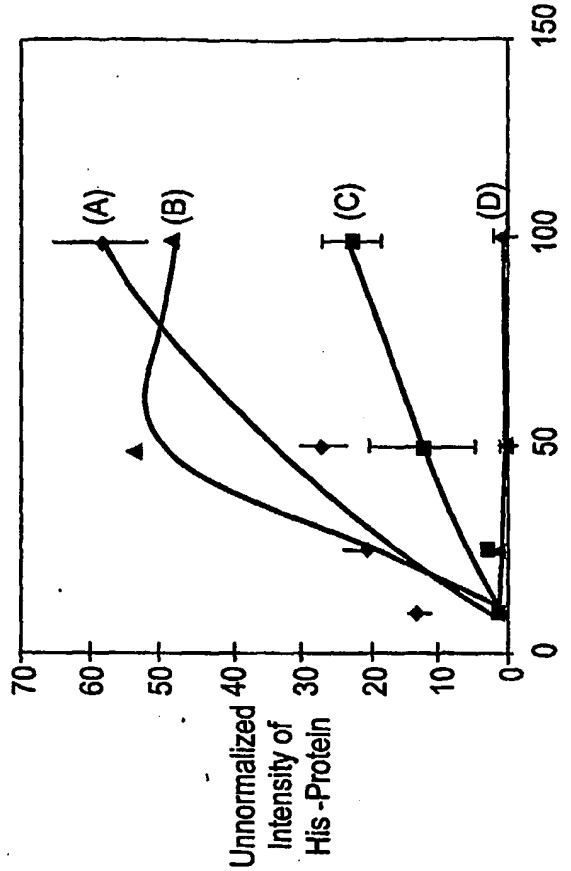
Expression of His-PelleC in *E. coli* directly on the surface of a ProteinChip™ Array. Induction using different concentration of IPTG. Expression up to 3 hrs.

FIG. 6B



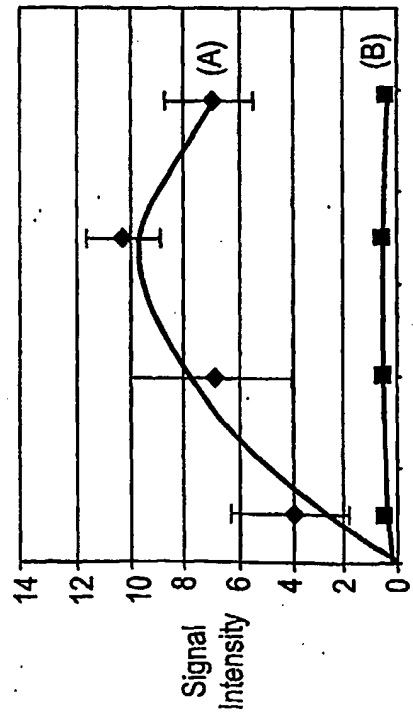
Schematic Diagram of Expression Device

FIG. 6A



Expression of His-PelleC in *E. coli* directly on the surface of a ProteinChip™ Array. Induction using 0.1mM IPTG. Expression time =2hrs. Comparison of disruption methods. (A) Centrifugation before Lysozyme disruption. (B) Lysozyme disruption without centrifugation. (C) Centrifugation only. (D) no centrifugation or Lysozyme.

FIG. 6D



Expression of His-PelleC in *E. coli* directly on the surface of a ProteinChip™ Array. Expression of protein with (A) or without (B) IPTG induction. Cells wre lysed directly in the media with the addition of Lysozyme.

FIG. 6C